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# Review article

## Insulin resistance and sarcopenia: mechanistic links between common co-morbidities

Mark E Cleasby<sup>1\*</sup>, Pauline M Jamieson<sup>2</sup> and Philip J Atherton<sup>3</sup>.

**1** Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, UK.

**2** Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, UK.

**3** Division of Medical Sciences and Graduate Entry Medicine, University of Nottingham, Medical School, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT, UK.

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**\*Corresponding author and address for reprints:** Dr Mark E Cleasby, Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, Royal College Street, London NW1 0TU. E-mail: [mcleasby@rvc.ac.uk](mailto:mcleasby@rvc.ac.uk). Tel +44 20 7468 5269. Fax +44 20 7467 5204.

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## Abstract

Insulin resistance (IR) in skeletal muscle is a key defect mediating the link between obesity and type 2 diabetes, a disease that typically affects people in later life. Sarcopenia (age-related loss of muscle mass and quality) is a risk factor for a number of frailty-related conditions that occur in the elderly. In addition, a syndrome of “sarcopenic obesity” (SO) is now increasingly recognised, which is common in older people and is applied to individuals that simultaneously show obesity, IR and sarcopenia. Such individuals are at increased risk of adverse health events versus those who are obese or sarcopenic alone. However, there are no licensed treatments for sarcopenia or SO, the syndrome is poorly defined clinically and the mechanisms that might explain a common aetiology are as yet not well characterised. In this review, we detail the nature and extent of the clinical syndrome, highlight some of the key physiological processes that are dysregulated and discuss some candidate molecular pathways that could be implicated in both metabolic and anabolic defects in skeletal muscle, with an eye towards future therapeutic options. In particular, the potential roles of Akt/mammalian target of rapamycin signalling, AMP-activated protein kinase, myostatin, urocortins and vitamin D are discussed.

## **Associations between obesity, diabetes and skeletal muscle aging**

The International Diabetes Federation has estimated that there were 382 million people living with diabetes in 2013, with this number predicted to rise to 592 million by 2035, of which the significant majority would be of >40 years old (IDF 2013). Of these, 90% suffer from Type 2 diabetes (T2D), which is characterised by both beta-cell failure and resistance to the actions of insulin at the tissue level (insulin resistance; IR). As skeletal muscle is responsible for the majority of the body's post-prandial glucose disposal, IR in this tissue results in substantial whole body metabolic disturbances. However, it is likely that the metabolic disturbances associated with T2D are further exacerbated by the marked loss of skeletal muscle mass that can also be associated with these conditions (Kim, et al. 2010; Park, et al. 2009). Specifically, loss of muscle mass induces a 2-3% decline in basal metabolic rate per decade after age 20 and 4% per decade after age 50, resulting from concomitant loss of mitochondrial volume density and oxidative capacity (Conley, et al. 2000).

This loss of muscle mass in the elderly is also the principal factor responsible for "frailty", a syndrome that has been clinically defined as the possession of three of: unintentional weight loss (10 pounds (~4.5kg) in the past year), self-reported exhaustion, weakness (poor grip strength), slow walking speed and low physical activity (Fried, et al. 2001). Loss of muscle mass (atrophy) is an inevitable, although somewhat modifiable, process that occurs with aging (Sayer, et al. 2008), when it is referred to as primary sarcopenia. In contrast, secondary sarcopenia can result from reduced physical activity or pathological causes, such as cachexia associated with malnutrition, organ failure, inflammatory disease, malignancy or endocrine disease (Cruz-Jentoft, et al. 2010). Sarcopenia has been implicated as a risk factor for numerous adverse health outcomes associated with frailty, including weakness, falls and fractures, immobility, functional decline, disability and loss of independence in the elderly (Batsis, et al. 2014; Cruz-Jentoft et al. 2010). It has also been associated with increased mortality in some prospective studies (Landi, et al. 2013) but not others (Cesari, et al. 2009).

The concept of "sarcopenic obesity" (SO) was introduced to highlight a syndrome present in a group of older patients in whom obesity is accompanied by sarcopenia and IR (Baumgartner 2000). The prevalence of SO in a recent study of adults in the USA was estimated to be 18% of women and 42% of men of mean age ~70, with increased risks of mortality being demonstrated for either obese or sarcopenic women (Batsis et al. 2014). However, the significance of concurrent obesity and sarcopenia was really emphasised by a separate study of older people, which demonstrated a 2-3 times higher risk of developing disability associated with reduced activities of daily living in individuals with SO versus others with sarcopenia or obesity alone (Baumgartner, et al. 2004). Currently, the characterisation of SO is as yet confined to a group of clinical and epidemiological observations, rather than being underpinned by defined common mechanisms (Bollheimer, et al. 2012). Nevertheless, the apparently high prevalence of SO and its profound consequences for healthcare provision mandates that additional research is carried out into the mechanisms underpinning the syndrome, in order to establish whether the muscle loss and IR associated with SO are indeed inevitable co-morbidities and to identify more effective therapies. The recommended therapeutic interventions are confined to lifestyle changes and are of limited effect, as there are no currently licenced medications for the treatment of sarcopenia (Bouchonville and Villareal 2013).

In this review, we intend to highlight potential mechanisms and pathways that might underpin both sarcopenia and IR in aging muscle, which may in the future be of interest as therapeutic targets for SO.

## **Clinical and functional delineation of sarcopenia and sarcopenic obesity**

The study of sarcopenia is still hampered by a lack of consensus regarding both definitions and techniques for assessment. Various diagnostic criteria have been used in studies to date, but frequently

these have been established purely on Gaussian distributions of measurements made in the test populations (Baumgartner, et al. 1998; Janssen, et al. 2002; Newman, et al. 2003). More recently, two consensus statements have been issued aimed at defining sarcopenia objectively. The European Working Group on Sarcopenia in Older People stipulated that low muscle mass and either low muscle strength or physical performance should be present for a positive diagnosis to be made (Cruz-Jentoft et al. 2010), while the Society of Sarcopenia, Cachexia and Wasting Disorders defined “Sarcopenia with limited mobility” as lean appendicular mass/height<sup>2</sup> of 2 SDs or more below the mean for 20-30 year olds, with a walking speed of  $\leq 1\text{m/s}$  (Morley, et al. 2011).

In addition to the challenges of defining SO, its assessment may be confounded by unchanging or increasing body mass index in older individuals due to increased adiposity, as this will mask any coincident loss of skeletal muscle mass. Therefore, evaluation of SO necessitates the careful assessment of body composition by other methods (Muller, et al. 2012). For example, in a recent cross-sectional survey that considered risk factors for and associations with SO in Korean people >65 years of age, sarcopenia was defined as weight-adjusted dual x-ray absorptiometry-determined appendicular skeletal muscle mass <2 standard deviations below the mean for healthy young adults (Ryu, et al. 2013). In the separate longitudinal Korean Sarcopenic Obesity Study, the extent of visceral obesity at the start of the study was shown to correlate with the extent of loss of appendicular muscle over ~2 years of follow-up, indicating that there may be a causal component to this association. However, baseline muscle mass was unable to predict the development of obesity (Kim, et al. 2014).

A further challenge to the definition and assessment of SO is that loss of muscle strength with age is substantially more pronounced than loss of mass, suggesting that the close relationship between muscle cross-sectional area and mass in younger people is not maintained in sarcopenia (Klein, et al. 2002). Moreover, this notion asserts that the loss of skeletal muscle quality is a significant contributor to age-related frailty (Goodpaster, et al. 2006). Therefore, the term “dynapenia” has been proposed as a more clinically relevant alternative to sarcopenia, to reflect the fact that loss of muscle function and mass are not reciprocally related, and that the former is more relevant to increased risk of adverse events, such as falls (reviewed in (Manini and Clark 2012)). Indeed, using a tertile-based classification of both muscle strength and adiposity in a small study population, it was shown that presence of “dynapenic obesity” but not SO was predictive of increased risk of falls (Scott, et al. 2014). However, as sarcopenia and SO are the terms that are best established in clinical use (Cruz-Jentoft et al. 2010), we will utilise these in this review. A general summary of the factors involved in SO is presented as **Figure 1**.

Although numerous animal models have been established to study muscle atrophy associated with disuse (Bodine, et al. 2001a), denervation (Muller, et al. 2007), sepsis (Breuille, et al. 1998), cancer cachexia (Temparis, et al. 1994) and glucocorticoid administration (Gardiner, et al. 1980), it seems that sarcopenia associated with aging is mechanistically distinct from the acute atrophy induced by such disease processes (Edstrom, et al. 2006). Furthermore, the study of *bona fide* sarcopenia in animal models is hampered by the length of time animals must be housed in order to reach an age at which it is detectable (20-24 months for rodents) (Bernet, et al. 2014; Bollheimer et al. 2012; Fry, et al. 2015; Muller et al. 2007; Tardif, et al. 2014)). In addition, studies of animal models of SO demonstrating pathophysiological or molecular mechanisms pertinent to the development of the syndrome in humans have rarely been reported. However, some researchers have studied aged rats with diet-induced obesity (Bollheimer et al. 2012; Tardif et al. 2014), while obese Zucker rats are characterised by marked obesity, IR and generalised muscle atrophy (Nilsson, et al. 2013) and thus may be useful for study of SO at a younger age.

## **Insulin resistance with respect to skeletal muscle glucose, lipid and protein metabolism**

Peripheral glucose utilisation is reduced as part of the IR that develops with age (Gumbiner, et al. 1992) and is substantially impaired in T2D (Cusi, et al. 2000), but protein turnover is also dysregulated. Skeletal muscle accounts for 40-50% of lean body mass in an adult human and therefore for the majority of whole body insulin-stimulated glucose disposal (Baron, et al. 1988; DeFronzo and Tripathy 2009). Thus, muscle mass is an important determinant of glucose and energy homeostasis (Wolfe 2006), and is determined by the balance between protein synthesis and breakdown in the tissue. An abundant supply of essential amino acids both inhibits proteolysis and stimulates protein synthesis (Castellino, et al. 1987; Cuthbertson, et al. 2005; Giordano, et al. 1996), while at least in younger people, insulin has a predominant effect to inhibit protein catabolism in muscle (Abdulla, et al. 2016; Fukagawa, et al. 1985; Gelfand and Barrett 1987).

Insulin-mediated accretion of muscle mass has been ascribed to activation of p38 mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR)/p70S6 kinase, and thus stimulation of mRNA translation (Fujita, et al. 2007; Guillet, et al. 2004a; Kimball, et al. 1998). In humans it is most likely that these effects are mediated through enhanced amino acid availability or delivery through increased perfusion (Fujita, et al. 2006; Timmerman, et al. 2010), all of which have been reported to be impaired in aged muscle (Bell, et al. 2005; Cuthbertson et al. 2005; Groen, et al. 2014; Rasmussen, et al. 2006). Thus the concept of age-related “anabolic resistance” has been proposed, to describe the reduced muscle protein synthesis that occurs in response to nutrients (Cuthbertson et al. 2005) or insulin (Fujita, et al. 2009; Rasmussen et al. 2006) and the reduced insulin-mediated suppression of proteolysis (Guillet, et al. 2004b; Wilkes, et al. 2009) that is associated with sarcopenia.

Interestingly, resistance to the anabolic action of insulin has been demonstrated in older individuals of normal muscle mass, and may therefore precede the physical manifestations of sarcopenia (Rasmussen et al. 2006). Indeed, it seems that differential IR with respect to glucose, protein and lipid metabolism can develop with aging, IR and SO. For example, many older individuals are insulin sensitive with regard to glucose metabolism, but not protein synthesis (Fujita et al. 2006). However, insulin, essential amino acids and resistance exercise are all less effective at inducing increases in muscle protein synthesis with increasing adiposity (Guillet, et al. 2009; Murton, et al. 2015; Nilsson et al. 2013). Metabolite fluxes within young, normal muscle and in muscle from older SO patients are summarised in **Figure 2**.

Adding further complexity, muscles of differing fibre type composition show contrasting sensitivity of both glucose and protein metabolism to insulin (Baillie and Garlick 1991; Lillioja, et al. 1987). T2D is characterised by reduced numbers of predominantly oxidative type I fibres relative and more glycolytic type II fibres (Oberbach, et al. 2006), with the proportion of type I fibres correlating positively with insulin sensitivity (Stuart, et al. 2013). Aging also results in a preferential reduction in the size of type II fibres (Lexell 1995), and the net result is that reduced mitochondrial activity (Johannsen, et al. 2012) and IR (Groen et al. 2014; Tardif et al. 2014) may also be evident in muscle. In summary, it appears IR, loss of muscle mass and changes in muscle fibre type all have the potential to independently or additively alter whole body glucose homeostasis with aging.

Clearly, defects that impair insulin-stimulated glucose disposal into muscle and thus negatively impact on whole-body glucose homeostasis will likely be compounded by concurrent sarcopenia, as in SO. It is known that interventions aimed at increasing muscle mass counter the development of IR (Dela, et al. 1996), but it is still not fully appreciated whether this is merely due to a proportionate increase in capacity for glucose disposal, or whether metabolic adaptation works synergistically with an increase in muscle mass. Recent studies in our laboratories have illustrated the potential for a dual effect, as manipulating bioavailability of single proteins in individual muscles, for example by inhibition of myostatin (Cleasby, et al. 2014), can result in enhanced glucose disposal on a per unit mass basis in addition to increased muscle mass, and therefore an enhancement in the total capacity for glucose disposal into the tissue.

## **Possible mechanisms: Accumulation of intramyocellular lipid and intermuscular adipocytes**

Both aberrant adipogenesis in muscles and excess intracellular lipid deposition have been associated with impaired muscle mass and insulin sensitivity. Increased adipocyte infiltration between muscle fascicles has been associated with both impaired gait (Scott, et al. 2015) and IR (Albu, et al. 2005). Furthermore, a longitudinal study demonstrated that progressive loss of muscle mass/quality was associated with increasing intermuscular fat in both aging humans (Delmonico, et al. 2009) and rats (Tardif et al. 2014), while another recent paper has shown that cultured intermuscular adipocytes produce pro-diabetic substances, providing evidence of a causal relationship (Laurens, et al. 2015). Additionally, a mechanistic link between expansion of visceral adipose tissue and muscle atrophy has been suggested by the observation of reduced expression of contractile proteins in human myotubes co-cultured with visceral adipocytes from obese subjects (Pellegrinelli, et al. 2015).

The impact of accumulation of intramyocellular lipid (IMCL) has been thoroughly studied and there is a well-established association between IMCL and muscle IR and T2D. However, triacylglycerol, the main storage form of lipid, is not thought to be mechanistically linked with the development of IR (reviewed in (Turner, et al. 2014)). Instead, the more bioactive derivatives ceramide and diacylglycerol have direct inhibitory effects on insulin signalling and metabolism (Chibalin, et al. 2008; Ussher, et al. 2010). Increased IMCL has also been associated with impaired muscle function in a number of studies. Lipid infusion results in reduced protein synthesis in response to both amino acids and insulin in healthy human volunteers (Stephens, et al. 2015), while diet-induced obesity and ectopic deposition of lipid in muscle rather than adipose tissue is also associated with impaired protein synthesis in rodents (Anderson, et al. 2008; Masgrau, et al. 2012; Tardif et al. 2014). This is associated with increased phosphorylation of elongation factor 2B, a key mediator of ribosomal protein synthesis, in rodent muscle, and a saturated fatty acid (SFA)/ceramide-induced increase in elongation factor 2 $\alpha$  activation in cultured muscle cells (Tardif et al. 2014). However, the nature of the lipids is important, because diets enriched in the SFAs impair muscle protein synthesis in rats than those based on unsaturated fatty acids (Tardif, et al. 2011), in addition to their increased tendency to cause insulin resistance (Budohoski, et al. 1993). The effect of increased IMCL on metabolite fluxes in muscle of sarcopenic obese patients is summarised in **Figure 2**.

## **Inflammation in obesity and in muscle**

Obesity is now recognised to be a subclinical inflammatory state, characterised by increased infiltration of adipose tissue with pro-inflammatory cell types, most notably macrophages (Lumeng, et al. 2007). Macrophage infiltration has also been demonstrated by a number of groups (Fink, et al. 2014; Hevener, et al. 2007), but not all (Tam, et al. 2012), to be a feature of obesity-associated IR in skeletal muscle and a synergistic interaction between macrophages and fatty acids that leads to impaired muscle insulin action has been reported (Varma, et al. 2009). However, an alternative proposal is that the dyslipidaemia associated with obesity activates cellular stress signalling pathways and thereby apoptosis and atrophy in skeletal muscle (Sishi, et al. 2011). In particular, SFA specifically can induce pro-inflammatory macrophage activation and consequent p38 MAPK-mediated IR in cultured myotubes, an effect that is ameliorated by the UFA palmitoleate (Talbot, et al. 2014). This role of p38 MAPK contrasts with its positive involvement in normal insulin-stimulated glucose disposal into muscle (Kimball et al. 1998), while in addition, loss of skeletal muscle satellite cell self-renewal is associated with impaired p38 MAPK  $\alpha/\beta$  activation in aged muscle (Bernet et al. 2014), implying that non-specific inhibition of this kinase is unlikely to yield overall beneficial effects *in vivo*. The explanation for these apparently disparate roles of p38 MAPK may be distinct functional specificities of the four identified isoforms of the kinase (Brault, et al. 2013), a possibility that has as yet not been fully investigated.

Further evidence implicates the balance between M1 and M2-type macrophage levels in muscle function. Obesity is characterised by accumulation of M1-type macrophages, at the relative expense

of the M2 subtype (Lumeng et al. 2007). However, muscle expression of M1-related cytokines correlates positively with muscle mass and strength (Beenakker, et al. 2013), while M2a-type macrophages accumulate in aging muscle (Wang, et al. 2015). Thus, the shift in macrophage phenotype with aging may be in the opposite direction to that in insulin resistant muscle.

Skeletal muscle inflammation is also characterised by activation of the classical signalling pathway to the transcription factor Nuclear Factor  $\kappa$ B (NF $\kappa$ B). Chronic activation of this pathway causes profound atrophy in mouse muscle (Cai, et al. 2004), while correspondingly it is activated by immobilisation of muscle (Bar-Shai, et al. 2005) and targeted ablation of the NF $\kappa$ B activating enzyme Inhibitor  $\kappa$  B kinase 2 (I $\kappa$ K2) improves skeletal muscle strength, maintains mass and promotes regeneration (Mourkioti, et al. 2006). However, short-term muscle fibre-specific overexpression of I $\kappa$ K2 or the p65 subunit of NF $\kappa$ B, sufficient to cause atrophy, does not impair insulin-stimulated glucose disposal (Polkinghorne, et al. 2008), providing further evidence that these two phenotypes are not inextricably linked as part of a pro-inflammatory phenotype.

### **Other molecular pathways potentially mediating the development of both sarcopenia and insulin resistance**

A summary of the roles of the molecules and pathways discussed here in glucose and protein metabolism is presented as **Figure 3**.

#### *Phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signalling*

Insulin and insulin-like growth factor-1 (IGF1) have predominantly metabolic and anabolic effects on muscle, respectively. However, upon binding to their cognate receptors, both exert their effects by recruitment of intracellular adaptor proteins, including insulin receptor substrate 1, to the receptor complex, and activation of Phosphatidylinositol 3-kinase (PI3K). The resulting phosphoinositol triphosphate promotes phosphorylation of protein kinase B/Akt, which then phosphorylates various substrates that orchestrate the various physiological effects of the two hormones. Increased glucose disposal is mediated predominantly through phosphorylation of Akt substrate of 160kDa (TBC1D4) and TBC1D1, and thus movement of GLUT4-containing vesicles to the plasma membrane (Cartee and Funai 2009), as well as disinhibition of glycogen synthesis by phosphorylation of glycogen synthase kinase 3. Akt-mediated activation of mTOR, and thus p70S6 kinase and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), is responsible for protein synthesis, and indeed amino acid-stimulated protein synthesis is also mediated through activation of mTOR. In parallel, Akt-mediated inhibition of forkhead transcription factor (FOXO) activity reduces expression of the E3 ubiquitin ligases that are principally responsible for mediating atrophy (atrogin-1/ muscle atrophy F-box and muscle RING finger 1) (Schiaffino, et al. 2013).

Consequently, activation of proximal PI3K pathways would be expected to have dual positive effects on muscle size and metabolism. This is clearly illustrated by muscle overexpression of Akt in rodents, which causes both muscle hypertrophy and increased glucose disposal per unit muscle mass, with the predominant effects determined by the isoform used (Akt 1 versus Akt 2-predominant effects, respectively) (Bodine, et al. 2001b; Cleasby, et al. 2007). Furthermore, defects in both components of the pathway were identified in leptin receptor-null (db/db) mice and obese Zucker rats. Treatment with the insulin sensitising thiazolidinedione drug rosiglitazone also led to an improvement in muscle mass, leading to the suggestion that IR *per se* could cause muscle wasting through suppression of PI3K/Akt signalling (Katta, et al. 2010; Wang, et al. 2006). However, it is equally plausible that activation of the Akt-mTOR cellular signalling pathways following peroxisome proliferator-activated receptor (PPAR) $\gamma$  stimulation by rosiglitazone impacts positively on both IR and muscle mass. The physiological relevance of this is unclear, as impairment of the Akt-mTOR pathway in muscle does not seem to occur naturally in aging humans or mice (Sandri, et al. 2013), but it may yet represent a therapeutic target.



## 290 *AMP-activated protein kinase*

291 AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated by an increased  
292 AMP:ATP ratio, leading to increased glucose and fatty acid uptake and oxidation in skeletal muscle  
293 (Koh, et al. 2008). It plays a major role in coordinating energy use during exercise in muscle, but also  
294 mediates the longer term effects of exercise, through mitochondrial biogenesis. This process is  
295 initiated by AMPK-mediated activation of silent mating-type information regulator 2 homolog 1  
296 (SIRT1) and PPAR coactivator-1 $\alpha$  (PGC1 $\alpha$ ) (Mounier, et al. 2015). AMPK has been extensively  
297 studied as a potential molecular target for the development of novel therapies for T2D (Coughlan, et  
298 al. 2014) and recent work has identified an additional role for AMPK in muscle turnover/plasticity. It  
299 can protect against age-related functional and mitochondrial impairment by promoting myocyte  
300 macroautophagy, an essential process for cellular maintenance (Bujak, et al. 2015). AMPK likely  
301 mediates the effects of adiponectin to promote macroautophagy (Liu, et al. 2015), which likely at least  
302 partly mediates this adipokine's insulin-sensitising effect in muscle (Patel, et al. 2012). However, the  
303 effects of AMPK on muscle mass appear less favourable. A study of aging rodents showed an inverse  
304 relationship between activating AMPK phosphorylation and load-induced hypertrophy (Thomson and  
305 Gordon 2005). Furthermore, AMPK stimulates myofibrillar protein degradation through increased  
306 FOXO expression (Nakashima and Yakabe 2007) and causes downregulation of the mTOR pathway,  
307 thus restricting protein synthesis (Bolster, et al. 2002). In addition, liver kinase b1 (Lkb1), one of  
308 AMPK's upstream kinases, has been shown to limit differentiation of satellite cells (stem cells present  
309 in adult skeletal muscle) through the same mechanism (Shan, et al. 2014). Thus, further studies are  
310 necessary to ascertain whether AMPK activation would have a net beneficial effect in individuals  
311 with both IR and sarcopenia.

## 312 *Myostatin*

313 Myostatin (MSTN) is now well-established as a central determinant of muscle size and mass, as  
314 demonstrated by the pronounced increases in muscle mass caused by gene-inactivating mutations in  
315 mice (McPherron, et al. 1997) and by naturally occurring genetic loss of function variants in several  
316 domestic species (Hill, et al. 2010). Consistent with this, its expression has also been shown to be  
317 increased in sarcopenia in some studies (Leger, et al. 2008), but this has not been a universal finding  
318 (Ratkevicius, et al. 2011).

319 However, in addition to its effects on muscle mass, MSTN deficiency has more recently been shown  
320 to have beneficial effects on metabolism, adiposity and insulin sensitivity. Both MSTN-null mice  
321 (Guo, et al. 2009) and mice treated either with the soluble MSTN receptor Activin Receptor IIb  
322 (Akpan, et al. 2009), which sequesters the mature peptide in the plasma, or the natural inhibitor  
323 follistatin-like 3 (Brandt, et al. 2015), show increased muscle glucose utilisation and insulin  
324 sensitivity, associated with increased lean mass and decreased fat mass.

325 Genetic or pharmacological inactivation of MSTN increases activation of AMPK (Zhang, et al. 2011),  
326 increases lipolysis and fatty acid oxidation in peripheral tissues, and also increases the expression of  
327 brown adipocyte markers in white adipose tissue (Zhang, et al. 2012), providing a number of potential  
328 mechanisms for its metabolic activity. Importantly, we have also recently shown that short-term local  
329 impairment of MSTN action in rats by overexpression of the myostatin propeptide and sequestration  
330 of the active peptide enhances skeletal muscle glucose disposal to a greater extent than would be  
331 expected due to increased muscle mass alone, implying that additive or synergistic mechanisms are in  
332 operation. The associated increase in glucose transporter (GLUT1 and GLUT4) protein levels may  
333 underpin the metabolic effects observed (Cleasby et al. 2014).

334 A number of modalities utilising inhibition of MSTN activity as a therapeutic approach have not yet  
335 borne fruit, although antisense-mediated destructive exon skipping is currently being evaluated. This  
336 has shown some promise in preserving muscle mass in a mouse model of Duchenne muscular  
337 dystrophy (Lu-Nguyen, et al. 2015), and its metabolic effects are currently under investigation.

## 338 *Urocortins*

339 Urocortins (Ucns) are neuropeptide ligands for the corticotropin-releasing factor receptor 2 (CRFR2)  
340 that are expressed not only in the central nervous system, but also in peripheral metabolic tissues.  
341 There are particularly high levels of Ucn2 and CRFR2 in skeletal muscle (Chen, et al. 2006), implying  
342 that these ‘stress regulators’ play a role in muscle physiology. Furthermore, CRFR2 expression was  
343 reduced on average by 71% and 92% in *soleus* and *tibialis cranialis* muscles, respectively, of aged  
344 mice (24 vs 3 month old; n=6, p<0.001).

345 Global knockout of either Ucn2 or CRFR2 produced mice that were resistant to diet-induced obesity  
346 and IR (Bale, et al. 2003; Chen et al. 2006), the former also demonstrating increased muscle mass.  
347 Interestingly however, global overexpression of Ucn3 also resulted in mice with marked muscular  
348 hypertrophy. These mice had increased IGF1 expression in muscle and also resisted the increased  
349 adiposity and metabolic abnormalities associated with feeding a high fat diet, despite the lack of  
350 endogenous muscle Ucn3 expression (Jamieson, et al. 2011). In order to dissect the muscle-  
351 autonomous component of this phenotype further and to indicate whether CRFR2 might have  
352 potential as a therapeutic target, we performed short-term overexpression of Ucn3 in rat muscle and  
353 showed increased glucose disposal, associated with elevated levels of glucose transporter expression,  
354 and phosphorylation of both AMPK and insulin signalling intermediates, before any increased muscle  
355 mass was detectable (Roustit, et al. 2014). Thus, a strategy to target CRFR2 also may have potential  
356 to improve muscle mass and metabolism additively.

## 357 *Vitamin D*

358 There has recently been renewed interest in potential novel roles for vitamin D (VitD), including in  
359 the maintenance of muscle mass and insulin sensitivity, which has been provoked in part by the  
360 identification of a high prevalence of VitD deficiency among adults (Bates, et al. 2011). Profound  
361 dietary insufficiency leads to impaired muscle strength as a result of hypophosphataemia in rats  
362 (Schubert and DeLuca 2010). However, epidemiological and intervention studies in humans have  
363 yielded contradictory results with regard to the role of VitD in muscle mass/function and metabolic  
364 endpoints. For example, insulin sensitivity has been reported to be either improved or unaffected by  
365 VitD supplementation (Talaie, et al. 2013; Wongwiwatthanakit, et al. 2013). VitD supplementation  
366 was reported to increase muscle fibre size in immobile older women (Ceglia, et al. 2013), but a recent  
367 systematic review of studies showed a benefit of supplementation for individuals with VitD  
368 deficiency at the start of the trial in terms of improved muscle strength, but not in muscle mass or  
369 power (maximum force generated in minimum time) (Beaudart, et al. 2014). These contradictory  
370 findings might be a result of insufficient study power and imprecise subject selection in many  
371 instances.

372 Attempts to explain a hypothesised role for VitD in muscle on a molecular level have been few to  
373 date, but knockout of the VitD receptor (VDR) in mice resulted in reduced muscle size, impaired  
374 motor activity (Burne, et al. 2006) and abnormal muscle development (Endo, et al. 2003). In addition,  
375 VDR-null mice are leaner (Narvaez, et al. 2009), possibly due to increased uncoupling protein  
376 expression (Wong, et al. 2009), but conversely have recently been shown to be insulin resistant, a  
377 phenotype that was shown to be mediated through increased muscle FOXO1 activation (Chen, et al.  
378 2015). Further work is needed to define the mechanistic links between VitD, the VDR and aging-  
379 related phenotypes.

380

## 381 **Additional therapeutic perspectives**

382 Sarcopenia (Baumgartner, et al. 1999; Genton, et al. 2011; Lee, et al. 2007; Park, et al. 2010; Raguso,  
383 et al. 2006; Scott, et al. 2011; Szulc, et al. 2004) and SO (Ryu et al. 2013) have been associated with  
384 low levels of physical activity in both cross-sectional and longitudinal studies, while exercise-based

interventions are well established to improve both muscle mass and performance (Skelton, et al. 1995; Vincent, et al. 2002) and insulin sensitivity (Fujita et al. 2007) in aged individuals. However, it is clear that these interventions are of more use in the prevention, rather than treatment of sarcopenia or SO and metabolic dysfunction, as elderly individuals are often too frail to undertake the degree of exercise required to achieve a beneficial effect (Wolfe 2006), while they may also suffer from anabolic resistance.

In addition, it is clear that a profound reduction in dietary energy intake can have a remarkable effect to alleviate IR and T2D (Steven and Taylor 2015). However, the inevitable lean tissue mass that is lost using this approach alone renders it undesirable in the already sarcopenic elderly, unless concurrent exercise or appropriate nutritional supplementation is undertaken (Verreijen, et al. 2015; Yoshimura, et al. 2014). Although a comprehensive assessment of dietary approaches is outwith the scope of this review, it is clear that motivation and compliance can frequently be a major limiting factor in the success of such lifestyle interventions (Evangelista, et al. 2003).

In terms of current pharmacotherapy, androgen replacement in hypogonadal men is effective in increasing muscle mass, but its use is as yet unproven in normal aging individuals, and is accompanied by undesirable side-effects (Giannoulis, et al. 2012). Nevertheless, androgen use may also be associated with an improvement in insulin sensitivity (Traish, et al. 2009). The development of selective androgen receptor-modulating therapies (SARMs) may help mitigate many of these side effects. Preclinical and Phase II trials of candidate drugs have demonstrated beneficial effects upon insulin sensitivity as well as on muscle mass and strength (Dalton, et al. 2011; Gao, et al. 2005; Min, et al. 2009).

One possible novel therapeutic approach might be to stimulate satellite cell activity and thus myofibre regeneration or replacement (Bernet et al. 2014), with the intention not only of improving muscle strength, but capacity for glucose disposal. However, satellite cell ablation in adult mice did not affect age-related sarcopenia in a recent study (Fry et al. 2015), implying that strategies aimed at stimulating their fusion or proliferation may not be effective. Furthermore, chronic activation of pathways triggering muscle growth, such as the IGF1-Akt pathway (Bellacosa, et al. 2005), involves the activation of known oncogenes, and thus the risk of tumour development.

PGC1 $\alpha$  is another molecular target that might have promise as a candidate for alleviation of both metabolic inefficiency and sarcopenia. This molecule is regarded as a key mediator of the beneficial effects of endurance exercise. Increased expression of PGC1 $\alpha$  in muscle improves metabolic fitness and prevents sarcopenia in aging mice (Wenz, et al. 2009), although it is unclear whether it promotes muscle strength in addition. Activation of PGC1 $\alpha$  has been shown to result in increased secretion of a novel hormone, irisin, which alleviates IR in mice (Bostrom, et al. 2012), although the significance of this finding for human biology has been questioned (Raschke, et al. 2013; Timmons, et al. 2012). Nevertheless, there is much interest in designing an “exercise mimetic” drug, based on such a molecular target, which would both improve muscle mass/function and metabolism, to tackle obesity-related metabolic disorders. However, it would seem unlikely that an approach aimed at targeting a single mediator would be successful in human trials.

## Conclusions and challenges for the future

This review has discussed current knowledge of the physiological and molecular mechanisms that govern both atrophy/sarcopenia and insulin resistance in skeletal muscle. We have aimed to highlight potential common ground between these mechanisms that could point to future development of novel therapies for SO in the elderly.

A number of challenges remain to address the deficiencies in our knowledge of this syndrome:

- 431 1. To establish a robust clinical definition of SO to enhance study design and thus permit improved  
432 comparability between clinical studies.
- 433 2. To establish whether sarcopenia and muscle insulin resistance are in fact inevitable co-morbidities,  
434 given the substantial overlap in the molecular pathways that are dysregulated in each.
- 435 3. To develop a more suitable animal model for SO to permit more practical mechanistic studies and  
436 preclinical therapeutic trials.
- 437 4. To further elucidate the key molecular pathways mediating both pathologies, permitting  
438 identification of molecular targets suitable for the development of combined therapies.
- 439 Addressing these priorities will hopefully provide a sounder footing from which to attempt more  
440 rational treatment of this common and debilitating condition.

441

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443 The authors declare that there is no conflict of interest that could be perceived as prejudicing the  
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## **Figure legends**

### **Figure 1: Clinical characterisation of sarcopenic obesity.**

Sarcopenia, obesity and insulin resistance increase in prevalence with advancing age. When individuals display several of the clinical signs listed, they may be defined as showing sarcopenic obesity. Dotted arrows indicate likely causative relationships and suggest that insulin resistance may be central to the syndrome.

### **Figure 2: Insulin resistance and “Anabolic resistance” in skeletal muscle and the role of intramyocellular lipid deposition.**

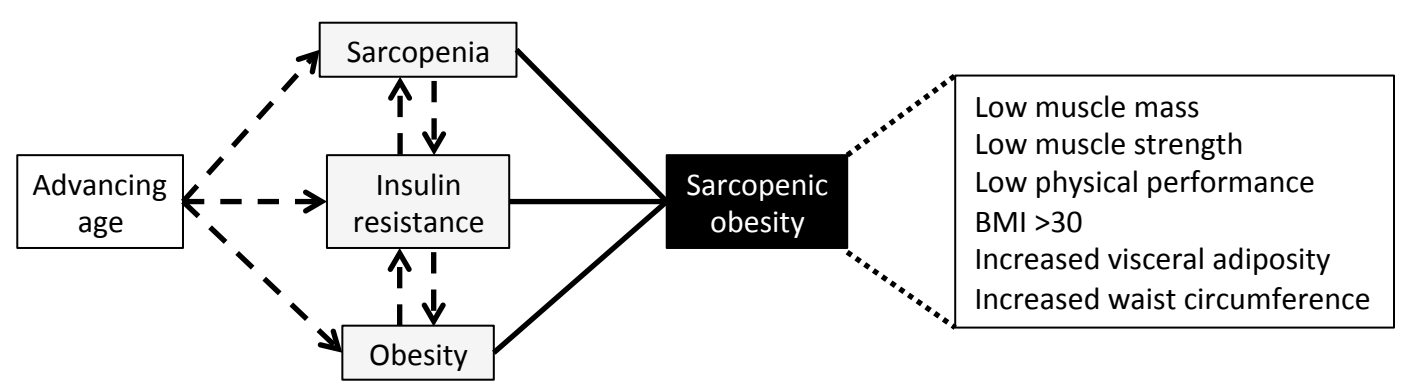
A- Normal muscle of young adult. Protein synthesis predominates over proteolysis under stimulation by supply of essential amino acids and insulin. Optimal insulin sensitivity favours glucose disposal and oxidation of lipids. B- Muscle of aged adult with sarcopenic obesity. Obesity-associated increases in intramyocellular lipid deposition, among other factors, causes impaired insulin signalling, protein synthesis and glucose metabolism. There is also a reduced anabolic response to exercise, amino acids and insulin. However, the extent of this resistance to insulin on protein, lipid and glucose metabolism varies between individuals. Straight arrows: metabolite flux. Broken straight arrows: reduced metabolite flux. Filled curved arrows: stimulatory effect. Open curved arrows: inhibitory effect.

### **Figure 3: Roles of selected candidate molecular mediators in skeletal muscle glucose and protein metabolism.**

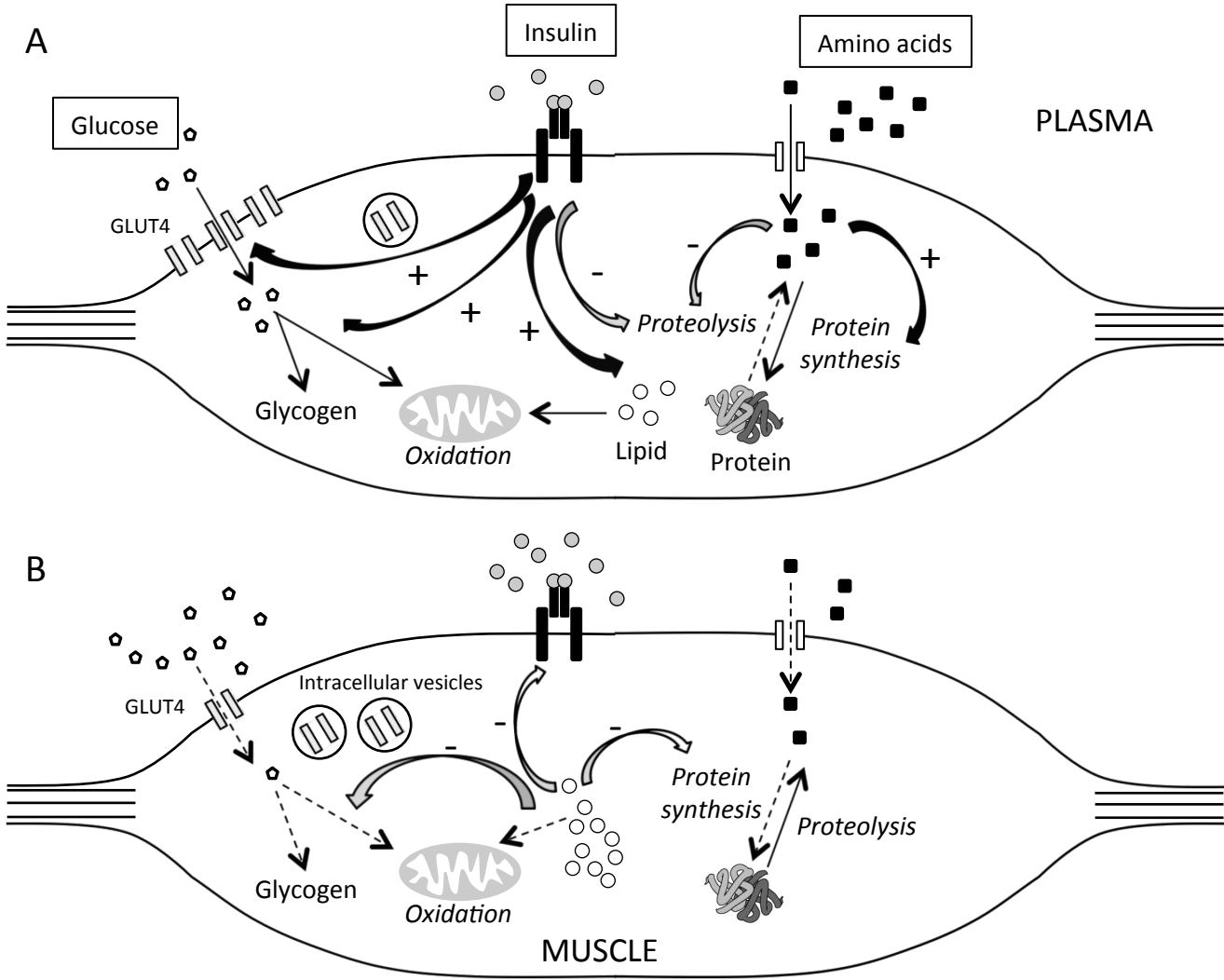
Published effects of insulin, insulin-like growth factor 1 (IGF1), amino acids, myostatin, urocortins and vitamin D on signalling pathways and effector machinery (glucose transporters, mitochondrial function, translation and activation of E3 ubiquitin ligases) relating to glucose and protein metabolism, as discussed in the text. Unmarked arrow: movement of molecules. Arrow with “+”: direct stimulatory effect on expression or activity. Arrow with “-”: direct inhibitory effect on expression or activity. Broken arrow: indirect effect. P indicates phosphorylation. Act2BR- Activin 2B Receptor. AMPK- AMP-activated protein kinase. AS160- Akt substrate of 160kDa. CRFR2- corticotropin releasing factor receptor 2. FOXO- forkhead transcription factor. GLUT4- Glucose transporter 4. IGF1R- IGF1 receptor. NFκB- Nuclear Factor κB. PGC1α- peroxisome proliferator-activated receptor coactivator 1α. MAPK- mitogen-activated protein kinase. mTOR- mammalian target of rapamycin. PI3K- phosphoinositol 3-kinase. SIRT1- sirtuin 1. VDR- vitamin D receptor.

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**Figure 3: Roles of selected candidate molecular mediators in skeletal muscle glucose and protein metabolism.** Published effects of insulin, insulin-like growth factor 1 (IGF1), amino acids, myostatin, urocortins and vitamin D on signalling pathways and effector machinery (glucose transporters, mitochondrial function, translation and activation of E3 ubiquitin ligases) relating to glucose and protein metabolism, as discussed in the text. Unmarked arrow: movement of molecules. Arrow with “+”: direct stimulatory effect on expression or activity. Arrow with “-”: direct inhibitory effect on expression or activity. Broken arrow: indirect effect. P indicates phosphorylation. Act2BR- Activin 2B Receptor. AMPK- AMP-activated protein kinase. AS160- Akt substrate of 160kDa. CRFR2- corticotropin releasing factor receptor 2. FOXO- forkhead transcription factor. GLUT4- Glucose transporter 4. IGF1R- IGF1 receptor. NFκB- Nuclear Factor κB. PGC1α- peroxisome proliferator-activated receptor coactivator 1α. MAPK- mitogen-activated protein kinase. mTOR- mammalian target of rapamycin. PI3K- phosphoinositol 3-kinase. SIRT1- sirtuin 1. VDR- vitamin D receptor.

